Impact of elevated atmospheric CO\textsubscript{2} concentration on pea and white melilot at three levels of nitrogen fertilization

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Abstract

Future environmental conditions will include elevated concentrations of nitrogen in the soils and elevated concentrations of carbon dioxide in the atmosphere. Increasing CO\textsubscript{2} concentrations are expected to enhance growth of agricultural C\textsubscript{3} crops. However, little is known about what are the consequences of a direct CO\textsubscript{2} fertilization’s effect for weeds and much more attention should be given to the combined effect of elevated CO\textsubscript{2} and N supply on plants. In order to study their interactions on both types of plant performance, growth chamber experiments were performed with C\textsubscript{3} crop pea (\textit{Pisum sativum} L.) and weed white melilot (\textit{Melilotus alba} Medik.) from the same family grown in a controlled conditions at different CO\textsubscript{2} levels (400 versus 700 and 1400 ppm) combined with three levels (3, 6 and 12 g/m\textsuperscript{2} of nitrogen) of fertilization. The photosynthetic rate, transpiration rate, stomatal conductance, water-use-efficiency and dry over-ground biomass were investigated at the end of an experiment after 10-day duration of treatment. Higher stimulatory effects of elevated 700 and 1400 ppm CO\textsubscript{2} concentrations were on photosynthetic parameters and growth of pea than of melilot. Contrarily, higher stimulatory effects of nitrogen supplies were on investigated parameters of melilot than of pea, but statistically significant only for transpiration rate, stomatal conductance and water-use-efficiency, at ambient and elevated CO\textsubscript{2} levels. The consistent response to both these factors identified in the plants was increased nitrogen use efficiency, who also revealed the dependence of the CO\textsubscript{2} response on N supply, as identified by a significant CO\textsubscript{2} × N interaction. According to these results, we concluded, that under future elevated CO\textsubscript{2} and nitrogen condition, both type of plants will be more efficient in resource use efficiency, but the ability of pea to assimilate additional carbon and the competitive advantage might increase more, compared to melilot.

Keywords: Elevated CO\textsubscript{2}; N fertilization; photosynthetic rate; growth; pea, white melilot.

1. Introduction

Agricultural plants with arable weeds cover more than half of Lithuanian territory. As for crop’s growth and productivity, as in alteration of weed competitions ability, environmental factors are very important. One of the most-recent global concerns of environmental issues is rapidly increasing levels of CO\textsubscript{2} in the atmosphere and its potential to change the world climate. CO\textsubscript{2} concentrations have been risen up from about 280 ppm in 1950 to about 385 ppm currently (~38% increase) [1]. Latest climate-change scenario projections predict that by the end of this century, CO\textsubscript{2} concentration will be in the range between 730 and 1020 ppm [2]. Because the current CO\textsubscript{2} is still below the saturation level of photosynthetic CO\textsubscript{2} uptake for C\textsubscript{3} plants, elevating CO\textsubscript{2} directly impacts photosynthetic processes in plants with the C\textsubscript{3} photosynthetic pathway evoking a wide range of physiological, biochemical and morphological responses. In generally, elevated CO\textsubscript{2} reduces transpiration by allowing plants to reduce their stomatal aperture while still receiving enough CO\textsubscript{2} for photosynthesis [3], resulting in an overall increase in water-use-efficiency (WUE) [4–8], and leading to a subsequent growth. There are a number of experiments with C\textsubscript{3} agricultural plants, showing that this group of plants would be induced favourably by elevated CO\textsubscript{2} concentration [4, 7, 9–15].

Interactions between crops and weeds, where a photosynthetic pathways differs, have been quite documented. The majority of them have focused on a C\textsubscript{4} crop in competition with C4 weeds, and generally, the results indicate that by CO\textsubscript{2} elevation, vegetative growth of C\textsubscript{4} crops is favoured relative to C4 weeds [5, 8, 16, 17]. In comparison of C4 crops with C\textsubscript{3} weeds, competitive ability of C\textsubscript{4} crops was decreased [17–19], consistent with the known biochemical response. However, fewer studies have examined weed/crop interactions for the same photosynthetic pathway at elevated CO\textsubscript{2} and; in those comparisons, available data are really different. In Ziska and Runion [5] represented summary of studies examining,
whether weed or crops were favored as a function of elevated CO$_2$, most comparisons with the same photosynthetic pathway for crops and weeds, either C$_3$ crop over C$_3$ weed or C$_4$ crop over C$_4$ weed, resulted in significant increases in weed-to-crop biomass, when weed and crop emerged simultaneously. On the other hand, in the study of Miri et al. [17] determined that C$_4$ crop (soybean) competitive ability (referred to plant relative yield measured as shoot dry weight in monoculture to shoot dry weight in mixed culture) decreased in the vicinity of C$_3$ weed (lambquarters), but C$_4$ crops (millet) competitive ability increased in the vicinity of C$_4$ weed (pigweed). However, when all species were grown in monoculture condition, crops both C$_3$ and C$_4$ responded better to CO$_2$ elevation than C$_4$ and C$_3$ weeds, respectively. Differently, in the experiment of Naidu and Varshney [20] assessed wheat (C$_3$ crop) had gained a competitive advantage over C$_3$ weeds under elevated CO$_2$, and it was observed that due to CO$_2$ enrichment the wheat plant could gain biomass against C$_3$ weeds, but that was variable with its association with different weeds. Similarly, Davis and Ainsworth [8] in an FACE experiment determined that elevated CO$_2$ strongly mitigated C$_3$ crop soybean yield loss due to weed interference by both C$_3$ and C$_4$ weed, however they found that elevated CO$_2$ did not affect weed growth rates, heights or final biomass and the reduction in weed survival under elevated CO$_2$ were slight and did not correlate significantly with soybean yield or yield loss due to weed interference, and speculated that elevated CO$_2$ may have mitigated weed interference with soybeans by reducing competition for soil moisture. Also in the Mini-FACE experiment of Erbs et al. [6], in comparison the CO$_2$ responses of species; it was found that spring wheat differed significantly (with the highest $A/g_s$, intrinsic WUE) from the CO$_2$ responses of arable weeds, when they were grown in an assembly under elevated CO$_2$.

Thus, data of the carried out experiments show that substantial variation in the response to elevated CO$_2$ exists even within the same photosynthetic pathway species, especially between weeds and crops and dependent on intercropping or monoculture conditions. Most of Lithuanian plants, because of climatic – meteorological environment in Lithuanian territory, is the C$_3$ photosynthetic pathway using plants, including major agricultural crops and dominant weeds. There are only few C$_4$ weeds among the most dominant and troublesome weeds in Lithuania and is only few C$_4$ agricultural species among major agricultural crops. And as was mentioned above, a little is known about what are the consequences of a direct CO$_2$ fertilization’s effect for weeds and much more attention should be given to the combined effect of elevated CO$_2$ and N supply on plants grown in Lithuania. So in this experiment there was investigated the effect of different levels (400 versus 700 and 1400 ppm) of CO$_2$ and three levels (3, 6 and 12 g/m$^2$ of nitrogen) of fertilization on C$_3$ crop pea (*Pisum sativum* L.) and weed white melilot (*Melilotus alba* Medik.) photosynthetic parameters and growth.

2. Methods

2.1. Experimental design and treatments

The experiments were conducted in closed controlled environment plant growth chambers located at Vytautas Magnus University, Lithuania in 2013. One C$_3$ crop – pea (*Pisum sativum* L. var. ‘Pinocchis’) and one C$_3$ weed – white melilot (*Melilotus alba* Medik.) of the same family were selected, as main nitrogen-fixing legume crop in Lithuania and dominant weed, respectively. Seeds of weed were collected in the arable field in autumn 2012. Uniform seeds of pea were selected and planted in 3 L plastic pots (diameter 21 cm, height 10.6 cm) containing a growth substrate composed of mixture of commercial peat (PROFI 2, pH 6.0, with a mixture of macronutrient-micronutrient) and fine sand (3:1 v/v). Seeds of weed were planted in 3 L plastic pots containing the same growth substrate too. The total concentration of nitrogen in the prepared growth substrate was determined using a method of Kjeldal, then was calculated the content of N in square meters of a growth area. So in a growth substrate there was 3 g/m$^2$ N content. The plants were grown in monoculture conditions (twenty individuals per pot) in a growth chamber with ambient 400 ppm CO$_2$ concentration. Chamber was 2.5 m high, 2 m wide, and 2 m long in length. The growth chamber was controlled with 70/80% relative humidity and a photoperiod of 14 h at 21 °C/14 °C (day/night) of air temperature. A photosynthetically active radiation (PAR) of 200 $\mu$mol m$^{-2}$ s$^{-1}$ provided by “Philips MASTER GreenPower CG T” 600W lamps in combination with luminescence lamps. The pots in the chamber were watered sufficiently and regularly. All treatments were run in three replicates.

After germination of pea plants when the second true leaf pairs unfolded (BBCH 12) [21] all pots were divided into three groups: first group of pea plants were left without any additional fertilisation, i.e. nitrogen content in growth substrate was 3 gN/m$^2$; second group of pots were supplied with 3 gN/m$^2$ content of NPK (12-11-18) fertilizers, so the total nitrogen content in the growth substrate became 6 gN/m$^2$; and third group of pea plants were supplied with 9 gN/m$^2$ content of NPK (12–11–18) fertilizers, that the total nitrogen content in the growth substrate became 12 gN/m$^2$. The day-after additional fertilization the impact of elevated CO$_2$ was started, and pea plants with different fertilization norms were divided in to three groups: a) first group of pea plants was left in the chamber with ambient 400 ppm CO$_2$-concentration; b) second group was transferred in the growth chambers with elevated 700 ppm CO$_2$; b) third group were transferred in the growth chamber with elevated 1400 ppm CO$_2$. The elevated CO$_2$ levels were maintained 24 h/d until the final harvest. The CO$_2$ concentration into the chambers was manipulated automatically by controlling the amount of CO$_2$ gas injected using a CO$_2$ delivery system and chamber vents.

The pots with weed plants from the growth chamber with ambient CO$_2$ were transferred in the growth chambers with elevated CO$_2$ when the second true pair of weed leaves emerged similarly as it was recorded in the case of pea, and one day before impact of elevated CO$_2$ the same part of pots with weed plants were supplied with solutions of NPK fertilizers that containing the same content of N, as they were on pea plants. Each pot was rotated under the same growing condition every two days to minimize the effects of differences in light, air temperature and CO$_2$ concentration within the local environment.
2.2. Biomass content measurements

At the end of experiments, after 10-day duration of treatment, crop and weed plants were harvested and divided into shoots and roots. Dry over-ground biomasses of 10 plants per pot of each pot were recorded and values were averaged as the values of each replicate. Therefore, each data point in the figures represents the mean value of three replicates per treatment. For determination of dry weight, shoots were cut at the base and weighted and dried in an electric air-forced oven at 70 °C until a constant dry weight was obtained (at least 72 hours).

2.3. Leaf gas exchange measurements

The measurements were made in a plant growth chambers and obtained from a closed infra-red gas analyzer LI-COR 6400 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE, USA). The time courses of actual single leaf photosynthesis (measured as \( A \), the rate of CO\(_2\) assimilation, \( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \)), transpiration rate (\( E, \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1} \)), stomatal conductance (\( g_s, \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1} \)) and water use efficiency (\( WUE, \mu \text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1} \)) recorded automatically simultaneously approximately five minutes (at 3-sec intervals), when a stable maximum \( A \) and maximum \( g_s \) were reached. After an equilibrium with the levels of CO\(_2\) used for growth was reached, the leaf was kept inside the assimilation chamber at constant conditions throughout all measurements using 160 \( \mu \)mol photons \( \text{m}^{-2} \text{ s}^{-1} \) photosynthetic active radiation (PAR), the vapor pressure deficit (VPD) at the leaf surface was approximately 1.5 kPa; the temperature in the six cm\(^2\) leaf chamber was between 22–25 °C, relative humidity (RH) of approximately 45% and growing CO\(_2\) concentration plants and 700 and 1400 \( \mu \text{mol CO}_2 \text{ mol}^{-1} \) for elevated CO\(_2\) concentration plants, respectively. Air flow rate through the assimilation chamber was maintained at 400 \( \mu \)mol s\(^{-1}\). The measurements of gas exchange were carried out between 10:00 a.m. and 5:00 p.m. on the last day of treatment for crop and weed. On each sampling date, assimilation was determined using fully expanded healthy leaves numbered fourth from plant bottom for each species in tree replicates (i.e. three plants per one CO\(_2\) treatment).

2.4. Statistical analysis

The independent-sample \( t \)-test was applied to estimate the difference between reference and treatment values. The levels of significance for differences between all measured physiological and morphological indices were analysed using factorial ANOVA. In all tests, CO\(_2\) treatment and sampling date were fixed effects and \( p \) value < 0.05 was the threshold for significance. All analyses were performed by \textit{STATISTICA} 8 and the results were expressed as mean values and their confidence intervals (\( p < 0.05 \) (± 95% CI)).

3. Results and discussion

The elevated 700 and 1400 ppm CO\(_2\) increased photosynthetic rate of pea and white melilot at all investigated nitrogen levels, and the increases of photosynthetic rate of pea plants were higher than the increases of melilot (Fig. 1.). Differences between nitrogen supplies under current climate conditions were higher for melilot, i.e. photosynthetic rate of melilot increased by 13% (\( p < 0.05 \)) and 16% (\( p < 0.05 \)) and photosynthetic rate of pea by 8% (\( p > 0.05 \)) and 12% (\( p < 0.05 \)), at 6 and 12 g/m\(^2\) N supply, respectively, compare to the 3 g/m\(^2\) N level. At elevated 700 ppm CO\(_2\) air condition statistically significant differences between nitrogen supply levels were only between 3 and 12 g/m\(^2\) nitrogen supply for melilot plants. At 1400 ppm CO\(_2\) air conditions photosynthetic rate of pea increased by 5.3% only at 12 g/m\(^2\) supply, compare to 3 g/m\(^2\).

![Fig. 1. Changes in photosynthetic rate of pea (a) and white melilot (b) plants under different CO\(_2\) concentrations and different nitrogen (N3, N6 and N12, i.e. 3, 6 and 12 g/m\(^2\)) content in growth substrate (mean ± 95% CI).](image-url)
Transpiration rate (Fig. 2.) and stomatal conductance (Fig. 3.) of both investigated plants decreased gradually under increasing CO$_2$ concentration, and the differences were higher for pea than for melilot. Differences of transpiration rate between nitrogen supplies under all investigated climate conditions were statistically significant, and the highest differences were detected at elevated 1400 ppm CO$_2$ concentration. The transpiration rate of pea plants grown at 1400 ppm CO$_2$ and 12 g/m$^2$ N supply was the lowest; also the lowest was detected stomatal conductance too. At elevated 1400 ppm CO$_2$ and 3, 6 and 12 g/m$^2$ nitrogen availability in growth substrate transpiration rate of pea decreased by 32%, 35% and 36%, and transpiration rate of melilot decreased by 9%, 14%, 14%, respectively, compare to the current climate conditions. If compare nitrogen supply effect on transpiration rate and stomatal conductance at different CO$_2$ conditions, the higher impact of N supply was detected for melilot than for pea. For example, at 700 ppm CO$_2$ stomatal conductance of melilot decreased by 9% ($p < 0.05$) and 14% ($p < 0.05$), and stomatal conductance of pea decreased by 7% ($p > 0.05$) and 12% ($p < 0.05$), at 6 and 12 g/m$^2$ N supply, respectively, compare to the 3 g/m$^2$ level. The higher availability of CO$_2$ decreased transpiration rate and stomatal conductance, but photosynthetic rate increased. As it was mentioned above elevated CO$_2$ reduces transpiration by allowing plants to reduce their stomatal aperture while still receiving enough CO$_2$ for photosynthesis [3], also resulting in an overall increase in water-use-efficiency (WUE) [7, 8]. In this research there was detected the same tendency, under increasing CO$_2$ concentration in the air, water-use-efficiency increased too (Fig. 4.). At elevated 700 ppm CO$_2$ and 3, 6 and 12 g/m$^2$ nitrogen supply WUE of pea increased by 79%, 78% and 78%, and WUE of melilot by 53%, 49%, 49%, respectively, compare to the current climate conditions. And at elevated 1400 ppm CO$_2$ and 3, 6 and 12 g/m$^2$ nitrogen supply WUE of pea increased by 151%, 149% and 148%, and by 63%, 59%, 59% of melilot, respectively, compare to the current climate conditions. As in case of photosynthetic rate, differences of WUE between nitrogen supplies under current climate conditions were higher for melilot, i.e. WUE of melilot increased by 18% ($p < 0.05$) and 28% ($p < 0.05$) under 6 and 12 g/m$^2$ N respectively, compare to the 3 g/m$^2$ N level, and WUE of pea plants increased by 12% ($p > 0.05$) and 22% ($p < 0.05$), under 6 and 12 g/m$^2$ N respectively, compare to the 3 g/m$^2$ N level. At elevated 700 and 1400 ppm CO$_2$ effect almost all WUE differences were statistically significant, compare to the 3 g/m$^2$ N supply, except WUE increase under N6 (6 g/m$^2$) nitrogen supply for pea plants at 700 ppm of CO$_2$. 

![Fig. 2. Changes in transpiration rate of pea (a) and white melilot (b) plants under different CO$_2$ concentrations and different nitrogen (N3, N6 and N12, i.e. 3, 6 and 12 g/m$^2$) content in growth substrate (mean ± 95%CI).](image1)

![Fig. 3. Changes in stomatal conductance of pea (a) and white melilot (b) plants under different CO$_2$ concentrations and different nitrogen (N3, N6 and N12, i.e. 3, 6 and 12 g/m$^2$) content in growth substrate (mean ± 95%CI).](image2)
The elevated 700 and 1400 ppm CO$_2$ increased dry biomass of both investigated plants, and the increases in biomass accumulation of pea plants were higher than that of melilot (Fig. 5). At elevated 700 ppm CO$_2$ conditions and 3, 6 and 12 g/m$^2$ nitrogen supply dry over-ground biomass statistically significant increased by 45%, 40% and 39% of pea, and by 32%, 24%, 24% of melilot, respectively, compare to the current climate conditions, and at 1400 ppm CO$_2$ concentration in air and 3, 6 and 12 g/m$^2$ nitrogen supply biomass accumulation statistically significant increased by 61%, 53% and 52% of pea, and by 36%, 25%, 25% of melilot, respectively, compare to the current climate conditions. If compare nitrogen supply effect on dry over-ground biomass at different CO$_2$ conditions the higher impact of N supply was detected for melilot than for pea. For example, at 1400 ppm CO$_2$ biomass accumulation of melilot increased by 0.7% ($p > 0.05$) and 2.0% ($p > 0.05$), at 6 and 12 g/m$^2$ N supply, respectively, compare to the 3 g/m$^2$ N level. So, as was mentioned above, there are a number of experiments with agricultural plants, showing that this group of plants would be induced favourably by elevated CO$_2$ concentration [7, 13–15]. Ziska and Runion [5] imply that similar benefits were likely for weedy competitors as well. However, it was shown that there is a great interspecific variation in plant to respond to CO$_2$ [22] and in the study of Archambault et al. [23] was found that responses of weeds and crops to increasing CO$_2$ levels were species-specific. Legumes, as a functional group, have the potential to respond most strongly to elevated CO$_2$, compared to other plant species [22, 24–26], because their N-fixing bacteria provide a large C sink where excess C can be traded for N allowing them simultaneously to avoid sink limitation and to increase their N supply [7, 25]. This regularity confirms results of this research too. CO$_2$ concentration in the air had the highest statistically significant impact on all investigated parameters, but also the changes of all parameters investigated also strongly depended on plants species (Table. 1.).
Because of greater range of responses observed for weeds to rising atmospheric CO$_2$, it was speculated that weeds have a greater genetic diversity and, hence, physiological plasticity, relative to crop species [5]. However, as revealed this study, weed was able to utilize fertilizers, as additional resource, more efficiently and less was able to the crop. But much more efficient in utilization of additional CO$_2$ was crop, relative to weed from the same family. Although investigated legume weed and crop showed somewhat different responses to the additional resources, one consistent response among the species to the increased CO$_2$ concentrations and nutrient supply was observed. For both species relative stimulation of increased CO$_2$ concentrations on plants growth were the larger, when the concentration of nitrogen in the growth substrate was the lower, and the impact of nitrogen for both type of plants growth was stronger at ambient than under elevated CO$_2$ concentrations. The percentage increases in both cases was mentioned above. These results indicate that elevated CO$_2$ allows increased efficiency of nitrogen use. An analysis of other evidence also shows that relative stimulation of plants grown with low N averaged across several studies appear just as large as those for plants grown with high N [27]. Also the enhanced N use efficiency for biomass (NUEp, ratio of biomass to cumulative N absorption) under elevated CO$_2$ was reported in rice [28, 29] and other species [30], assuming that the same amount of N taken up between elevated and ambient grown plants, greater dry matter will be produced under elevated CO$_2$ condition. It also revealed the dependence of the CO$_2$ response on N supply, as identified by a significant CO$_2$ × N interaction (Table. 1.). Because results from growth chamber studies cannot be extrapolated to the field, we are not sure about the ecological implications of the different growth responses to CO$_2$ and nutrient supply of the investigated legume crop and weed species. But it is expected that in long-run these would be detectable too, especially in competitive situation under progressively nutrient limited conditions.

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### 4. Conclusions

The controlled growth experiment data presented here shown that even the same photosynthetic pathway using and even from the same family crop and weed respond differently to CO$_2$ enrichment and nutrient supply. The different morphology and the investment in vegetative plant maters of different plant species, adaptation to the resource availability and the plasticity of individual plants, all these factors determine how a species will respond to global change. This study revealed that to the additional resources crop and weed from the same family responded in different ways. Weed was able to utilize fertilizers, as additional resource, more efficiently and less was able to the crop. But much more efficient in utilization of additional CO$_2$ was crop, relative to weed. Both investigated legume plants, pea and white melilot stronger responded to the elevated CO$_2$ than to nitrogen supplies. However higher stimulatory effects of elevated 700 and 1400 ppm CO$_2$ concentrations were on photosynthetic parameters and growth of pea than of weed white melilot. Contrarily, higher stimulatory effects of nitrogen supplies were on investigated parameters of melilot than of pea, but statistically significant only for transpiration rate, stomatal conductance and water-use-efficiency, at ambient and elevated CO$_2$ levels. Although investigated legume weed and crop showed somewhat different responses to the additional resources, one consistent species-specific response to the increased CO$_2$ concentrations and nutrient supply was observed. For both species effect of increased CO$_2$ concentrations on plats growth were the larger, when the concentration of nitrogen in the growth substrate was the lower, and the impact of nitrogen for both types of plant growth was stronger at ambient than under elevated CO$_2$ concentrations. These results shown that under impact of elevated CO$_2$ concentrations increased not only water-use-efficiency, but also nitrogen-use-efficiency increased too and confirmed that the primary effect of the response of plants to rising atmospheric CO$_2$ is to increase resource-use-efficiency. It also revealed the dependence of the CO$_2$ response on N supply, as identified by a significant CO$_2$ × N interaction. It is expected that in long-run these would be detectable too, especially in competitive situation under progressively nutrient limited conditions. According to these results, we confirmed that under future elevated CO$_2$ and nitrogen condition, both types of plant will be more efficient in resource-use-efficiency, but the ability of pea to assimilate additional carbon and the competitive advantage might increase more, compared to weed white melilot.
References


